REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1, 5, 7-14, 16-17 and 24 are pending.

The amendments are fully supported by the original disclosure and, thus, no new matter is added by their entry. The present claim 1 combines the limitations of previous claims 1 and 2, and is directed to "A method for determining coping capacity of a human or non-human mammal for exposure to a psychological stressor." Additional method steps are also recited. The term "coping capacity" is defined as responsiveness of a whole blood sample to induction of superoxide production by a chemical inducer which stimulates superoxide production in neutrophils (PMA is the chemical inducer n the examples). As clearly described in claim 1, a psychological stressor will itself induce superoxide production in neutrophils of a human or non-human animal susceptible to the stressor. An important aspect of Applicants' invention is to determine the residual capacity of neutrophils in whole blood samples to produce superoxide in the presence of an added chemical inducer as a measure of stress effect. It is imperative to note that measurements of superoxide production are made on whole blood samples (instead of isolated neutrophils) and the method requires comparison of chemically-induced super-oxide production above basal (i.e., without chemical inducer) in a test sample and a control sample. Step (b) requires determining basal superoxide production in the test sample in the absence of added chemical inducer. Step (e) requires a comparison of the superoxide production above basal in the test sample supplemented with inducer with the superoxide production above basal in a suitable control sample with added inducer. As specified at the end of claim 1, where the test sample is from a human or non-human animal subject to the effects of psychological stress, that sample will give lower super-oxide production above basal compared with the control. The degree of chemically-induced superoxide production above basal in the test sample will be a measure of coping capacity for exposure to the psychological stressor of interest: e.g., psychological stress in a human arising from taking a written test (see Example 4). This concept for quantifying the effect of a psychological stressor by a **simple whole blood test** relies on determining residual capacity of neutrophils for superoxide production in

whole blood samples. This limitation is nowhere taught or rendered obvious by the prior art cited in this application. Claims 12 and 16-17 are amended to be consistent with claim 1.

Claim 1 was objected to as allegedly informal. It is amended to correct the informality. The claimed methods are amended to limit their application to human and non-human mammals. The examples provided by Applicants in their specification are ample evidence that the method can be applied to either a human subject to art-recognized psychological stressors, such as exams and watching stressful events, or a non-human mammal exposed to psychological stress, such as through its handling and transport. Withdrawal of the objection is requested.

35 U.S.C. 112 – Written Description

The specification must convey with reasonable clarity to persons skilled in the art that applicant was in possession of the claimed invention as of the filing date sought. See *Vas-Cath v. Mahurkar*, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). But the Patent Office has the initial burden of presenting evidence or a reason why persons of ordinary skill in the art would not have recognized such a description of the claimed invention in the original disclosure. See *In re Gosteli*, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989).

Claims 1-2, 5-14, 16-17 and 23-24 were rejected under Section 112, first paragraph, as allegedly failing to comply with the written description requirement. In particular, it was alleged that definitions of the following limitations were not known and are not described in the present specification to allow one skilled in the art to practice the claimed invention: changed physiological status, psychological stressor, and basal. Applicants traverse.

The limitation "changed physiological status" is no longer recited because it is not required for patentability. Psychological stress is quantified by reference to a specific effect on neutrophils in the blood: i.e., induction of superoxide production. Applicants' invention determines the residual capacity of whole blood samples to produce superoxide when a chemical inducer is added. The examples show that the effectiveness of the chemical to produce superoxide is a reliable measure of the effect of psychological

stress. Their claimed method ensures that neutrophils and other leucocytes are kept in their natural blood environment where they are exposed to the complex biochemical changes in blood that arise from the experience of psychological stress. Significantly, there is no isolation of neutrophils required to obtain a known fixed number of isolated cells for use. This was a pitfall of prior art studies which looked at superoxide production from neutrophils in the presence of an inducer.

On page 4 of the Action, it appears that there was a failure to perceive that it is necessary to have control samples and to measure induced superoxide production at a level above a pre-measured basal level (with no inducer effect) for all samples (both control and test samples) to practice the claimed invention. As indicated above, present claim 1 recites as step (b) determining basal superoxide production in the test sample in the absence of induction of superoxide production by an added inducer. Step (c) recites determining superoxide production in the same sample plus inducer after a suitable time period and under suitable conditions. Step (d) recites determining how far superoxide production is boosted above the basal level in the test sample by the inducer. This measurement must be compared with the same measurement in a control sample (from a human or non-human animal not subject to the psychological stressor of interest). The role of the control sample is set out clearly by reference in steps (c) and (e).

Looking at Figure 1 of the specification, which represents the data from the study of Example 1, it can been seen that chemiluminescent assay measurement of superoxide was made at several time points in test samples (open squares) and controls (filled circles) after addition of inducer (PMA) at time zero. This time course illustrates that for convenience a single time point might be selected for measurements at which there is good induced chemiluminescence in the control sample, say 15 minutes. The amount of induction is seen by comparing the dotted lines, chemiluminescent measurement in the absence of effect of inducer (the basal level).

Turning to the beginning of Example 1 on page 15 of the specification, Applicants were interested in quantifying the added psychological effect on badgers stressed by their transport. Test whole blood samples were from badgers after trapping and transportation (cf. step (a) of claim 1). Control whole blood samples were taken from badgers

trapped but not transported (see bottom paragraph on page 15). Section (b) on page 16 specifies how the stress effect of transport was determined by PMA challenge. Basal (background) chemiluminescence after adding luminol to part of each sample (10 µl) was measured in a chemiluminometer (cf. step (b) of claim 1). The same initial blood samples (eight test samples and eight control samples) were used to measure chemiluminescence under the same conditions, except with addition of PMA. Looking at Figure 1, PMA induced relatively high chemiluminescence above basal in controls at 15 minutes. Looking at the same time point for test samples (cf. step (c) of claim 1), much lower chemiluminesence above basal was observed equating with lower PMA-induced superoxide production (cf. determining steps (d) and (e) of the claim 1). This permitted Applicants to confirm that transport did cause psychological stress as suspected. Moreover, the stress effect was such that PMA only poorly induced superoxide production. The psychological stress of transport had affected leucocytes including neutrophils such that there was little capacity for residual superoxide production. Such residual capacity of whole blood samples for superoxide production has been termed "leucocyte coping capacity" (LCC) and provides a quantitative measure of coping capacity for the psychological stressor of concern (cf. the conclusion of claim 1 and definition of "coping capacity").

Applicants also looked at LCC per 10^9 neutrophils/l and found the same overall pattern of results (page 18, second and third paragraphs). These were the preliminary studies which showed that they had indeed struck upon a simple whole blood test for truly quantifying stress. This is further supported by the other examples including data for humans exposed to stressful situations: e.g., humans who watched a horror film compared with controls who sat quietly without film watching. Again, basal superoxide without PMA was determined for both control whole blood samples and test whole blood samples taken at the same chosen time point for exposure of the test group to psychological stress.

Thus, the present claims clearly set out how to practice Applicants' invention to determine coping capacity of a human or non-human mammal for exposure to a psychological stressor as exemplified. There is no ambiguity whatsoever. The term

"basal" in the context of the examples and claims is used consistently and would be well understood by the person of skill in the art. One must have a basal measurement without inducer against which to judge whether superoxide has been induced by a chosen chemical inducer. In effect, there are two types of control: control whole blood samples and base line superoxide measurements for individual samples.

Withdrawal of the written description rejection is requested because the specification conveys to a person skilled in the art that Applicants were in possession of the claimed invention as of the filing date.

35 U.S.C. 112 – Enablement

The Patent Office has the initial burden to question the enablement provided for the claimed invention. M.P.E.P. § 2164.04, and the cases cited therein. It is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. *In re Marzocchi*, 169 USPQ 367, 370 (C.C.P.A. 1971). Specific technical reasons are always required. See M.P.E.P. § 2164.04.

Claims 1-2, 5-14, 16-17 and 23-24 were rejected under Section 112, first paragraph, because the specification allegedly does not reasonably provide enablement for determining a change in physiological status arising from any physiological stressor. Applicants traverse.

A person with knowledge of assays for superoxide would have no difficulty in applying a simple blood test as claimed to any situation where it is desired to assess the effect of psychological stress on a human or non-human mammal. Contrary to the Examiner's suggestion, the evidence on file shows that the beauty of the invention lies in its ease of application.

The working examples provide ample evidence that the invention is applicable to both humans and non-human mammals and that the test itself provides an internal control that a psychological stressor has been operative in a test subject or group. Any

doubt on the workability of the invention is directly contradicted by the evidence before the Examiner, including the additional information in the Mian Declaration.

Ellard used a histological staining technique to count activated cells. It showed that a short-term mental stressor causes an increase in the percentage of activated neutrophils. It is precisely because one cannot extrapolate from such studies anything useful about quantification of superoxide production that they cannot be compared with the invention. Example 4 of Applicants' specification shows application of the invention to humans subjected to a mental stressor task. This cannot be envisaged from Ellard's studies.

As set out in the response filed December 8, 2006 starting at page 24, Kang et al. employed for their PMA/FMLP-challenge assays isolated neutrophils subject to a lengthy handling procedure. The use of isolated neutrophils meant that superoxide production could be measured with a fixed number blood cells using a well-known colorimetric assay. It also meant that the neutrophils were subject to non-physiological conditions. It is therefore not surprising that the results in the subject specification are not comparable. The Kang et al. reference does not invalidate the Applicants' studies. Rather they highlight the importance of the invention. Kang was concerned about cell count. Instead, Applicants started from a different base and in so doing found that one could quantify the effect of psychological stress by keeping neutrophils in whole blood samples and simply assaying residual superoxide production capacity in the presence of a chemical inducer.

Further, it is emphasized that the nature of the invention (a whole blood chemical challenge test which may be carried out employing a conventional chemiluminescence assay for superoxide) means that it can be readily applied without any undue experimenttation. Arriving at the invention certainly required non-conventional thinking relying on a thought route missed even by experts in the stress analysis field. Kang well illustrates this. As emphasized previously, others were focuses on the change in neutrophil / leucocyte number. In contrast, the exemplification of the subject invention shows that neutrophils can be successfully used as biomarkers of psychological stress in whole blood samples whereby they are retained in their *in vivo* environment. Once this very

different concept was demonstrated, meaning that there was for the first time a truly physiologically relevant blood test for exposure to psychological stress, it was a far easier matter to apply to study of any recognized or suspected psychological stressor. All that is required is a simple blood test; it is wrong to suggest that there is anything more required beyond this. The blood test gives the answer as set out in the claims.

The invention works by focusing on one feature of the stress effect: superoxide production in blood – a reflection of changed physiological status brought on by exposure to psychological stress. As made explicitly clear in the present claim 1, what is quantified by the invention is residual capacity for superoxide production as determined by adding a chemical inducer. This reflects the effect of stress in a physiologically relevant manner and equates with the recognized conventional term "coping capacity." A person with a low coping capacity for a particular psychological stressor: e.g., stress as may arise in anticipating surgery or an exam, may exhibit little or no capacity for residual superoxide production compared to controls (not exposed to such stress). Their superoxide producing cells are exhausted by the stress effect itself.

The claimed invention has been already used to look at the effect of a diverse range of psychological stressors in a diverse range of subjects. Use has ranged from car design to housing of animals to sportsmen and women. This is evident from the information previously provided. There is every reason to believe that the method can be applied to at least any type of mammal. Necessarily the examples in the specification are illustrative of a selection of uses. The first interest of Applicants was wild animals (the inventor David MacDonald has a high interest in wildlife conservation). But after the initial study reported in Example 1 and published in McLaren et al. (2003), it was quickly realized that the invention was of far broader applicability and this is borne out by the further exemplification provided. If the invention works for badgers and humans, then there is every reason to believe that it can be applied broadly to humans and any type of non-human mammal in accordance with the new main claim.

In conclusion, the present claim 1 correctly reflects the enabled scope of the invention. There is no unpredictability in applying the invention. Applying the invention as claimed firstly provides direct confirmation that a psychological stressor has been

operative and secondly enables simple quantification of the stress effect (i.e., coping capacity for the stressor). That this is so is illustrated for both humans and non-human mammals by the many diverse examples now before the Examiner, both in the specification and provided in relevant journal papers and the Mian Declaration. The Mian Declaration attests that the invention in providing the first test for objectively assessing psychological stress is being actively used as a commercial test in a variety of situations.

Withdrawal of the enablement rejection is requested because it would not require undue experimentation for a person of skill in the art to make and use the claimed invention.

35 U.S.C. 112 – Definiteness

Claims 1-2, 5-14, 16-17 and 23-24 were rejected under Section 112, second paragraph, as being allegedly indefinite. Applicants traverse.

The limitation "same regime" is not recited because it is not required for patentability. The control sample is defined in step(c) in consistent manner with the exemplification. For any psychological stressor to be assessed, a person of skill would readily understand what is required by way of a control. Examples 1 and 4 well illustrate the use of controls as defined. In Example 1, the control samples were taken from badgers trapped but not transported where the psychological stressor of interest was transport after trapping. In Example 4, the control samples were taken from individuals sitting quietly away from exposure to any immediate stressful factor.

The claimed invention requires determining basal superoxide production in the test sample (see step (b) of claim 1). Steps (c) and (e) clearly explain how superoxide measurements on the test sample will be related to superoxide measurements on the control sample (minus inducer effect and at a time point after inducer addition at which the control exhibits induced superoxide induction).

Step (a) requires that the test whole blood sample is taken after exposure to said stressor for a time period whereby there is stress effect (equating with neutrophils being activated by the stress itself). This is confirmed by the test itself (see the conclusion of

claim 1) but might, if desired, be also be confirmed by other well known means (e.g., a conventional NBT neutrophil reduction test as is employed in Ellard's studies).

No essential steps are omitted because claim 1 includes all necessary features with corresponding steps for clear understanding by a person skilled in the art of how to perform the invention.

Applicants request withdrawal of the Section 112, second paragraph, rejection because the pending claims are clear and definite.

35 U.S.C. 103 – Nonobviousness

A claimed invention is unpatentable if the differences between it and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art. *In re Kahn*, 78 USPQ2d 1329, 1334 (Fed. Cir. 2006) citing *Graham v. John Deere*, 148 USPQ 459 (1966). The *Graham* analysis needs to be made explicitly. *KSR v. Teleflex*, 82 USPQ2d 1385, 1396 (2007). It requires findings of fact and a rational basis for combining the prior art disclosures to produce the claimed invention. See id. ("Often, it will be necessary for a court to look to interrelated teachings of multiple patents . . . and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue"). The use of hindsight reasoning is impermissible. See id. at 1397 ("A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning"). Thus, a prima facie case of obviousness under Section 103(a) requires "some rationale, articulation, or reasoned basis to explain why the conclusion of obviousness is correct." *Kahn*, 78 USPQ2d at 1335; see *KSR*, 82 USPQ2d at 1396. A claim which is directed to a combination of prior art elements "is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." Id. at 1396. Finally, a determination of prima facie obviousness requires a reasonable expectation of success. See *In re Rinehart*, 189 USPQ 143, 148 (C.C.P.A. 1976).

Claims 1-5, 9-11 and 23-24 were rejected as allegedly unpatentable over Mikawa (Can. J. Anaesth. 40:1162-1170, 1993) in view of Pfefferkorn (U.S. Patent 5,492,816). Applicants traverse.

Applicants' invention as claimed is a method for objectively assessing psychological stress. The present claim 1 explicitly requires that the method determine coping capacity for exposure to a psychological stressor (where coping capacity is defined as responsiveness of a whole blood sample to induction of superoxide production). The effect of psychological stress is quantified by the claimed method. It appears from statements made in the Office Action that there still exists many differences between the claims and how they are being construed by the Patent Office. In particular, the distinction between control samples and basal readings on test samples and control samples, which is essential for proper understanding of the invention, does not appear to have been correctly recognized during prosecution of this application. For example, Mikawa takes a second sample after general anesthesia and surgical treatment. This immediately precludes any relevance to determining effect of a psychological stressor. The invention concerns quantifying the effect of psychological stress. Psychological stress demands that the brain is aware of a stressor; the whole point of general anaesthesia is to make the patient unaware of pain and surroundings plus surgery itself affects the activation of neutrophils. Indeed, this is what Mikawa confirmed in neonates and infants. According to Applicants' invention, differences must be quantified in test (i.e., after exposure to a psychological stressor) compared to control (i.e., substantially free of stress-induced activation or at least derived from a subject to the same conditions minus the stressor) samples, and defining coping capacity as responsiveness of a whole blood cell sample to induction of superoxide production by a chemical inducer which stimulates superoxide production in neutrophils.

A psychological stressor will itself induce superoxide production in neutrophils of a human or non-human animal susceptible to the stressor. The essence of the invention is to determine residual capacity of neutrophils in a whole blood sample for superoxide production in the presence of an added chemical inducer as a measure of stress effect. It is imperative to note that measurements of superoxide production are made on whole

bloods samples (not isolated neutrophils) and the method requires comparison of chemically-induced superoxide production above basal (i.e., without inducer) in a test sample and a control sample. Where a test sample is from a subject to the effects of psychological stress, the sample will give lower superoxide production above basal compared with the control. The degree of chemically-induced superoxide production above basal in the test sample will be a measure of coping capacity for exposure to the psychological stressor of concern: e.g., psychological stress in a human arising from taking a written test (see Example 4). This concept for quantifying the effect of a psychological stressor by a **simple whole blood test** relying on determining residual capacity of neutrophils for superoxide production in whole blood samples is nowhere pointed to in the prior art of record.

Mikawa neither teaches nor renders obvious a procedure suitable for objectively assessing any effect of psychological stress. A sample was taken prior to anesthesia, but the next sample was taken was after surgery. This necessarily means that nothing can be gleaned from Mikawa's studies about the effect of psychological stress, which may have occurred prior to surgery. Mikawa's disclosure is only concerned with effects of surgical treatment. Surgery is physical trauma; it is incorrect to equate surgery with psychological stress. Psychological stress demands that the patient's mind is aware of the stressor, while the whole point of general anesthesia is to make the patient unaware of pain. No studies were done using test samples and appropriate controls designed to look at pre-surgical events as a psychological stressor. Mikawa was concerned with looking at how the entire experience of surgery affects neutrophil activity in neonates and infants against the hypothesis that this could be a contributor to the incidence of post-operative infection. The failure to obtain time points prior to surgery limits Mikawa's conclusions to the effects of physical stress (cf. Example 8 of the specification).

As already attested by an inventor Dr. Rubina Mian, "There is simply nothing in the disclosure of the Mikawa et al. paper which is remotely of interest" to the claimed invention (see Mian Declaration at paragraph 12). Mikawa's mention of PMA, neutrophils, and superoxide production does not make it relevant to the claimed invention. Not only is Mikawa's teachings solely confined to looking at the effect of surgical treatment

on neutrophils, it is also important to note that Mikawa chose to use a fixed quantity of isolated neutrophils for stimulation with inducer. Mikawa is not made any more relevant by its combination with Pfefferkorn because Applicants' invention is not simply a PMA-challenge assay with chemiluminescence measurement. Their invention as described in the present specification lies in the discovery that non-isolated neutrophils can be used as a biomarker in whole blood assays for objective assessment of psychological stress. Mikawa discloses that surgery affects leucocyte reactivity. This is interesting for those concerned with improving recovery from surgery, but had no bearing, and could have no bearing, on finding an improved way of assessing psychological stress.

Applicants' claims require the use of whole blood samples. The main argument with respect to Mikawa is not, however, centered principally on the type of sample. It is that Mikawa is not relevant prior art. A pre-operative period might give rise to psychological stress in certain individuals, but it is emphasized that Mikawa did not look at this. It is unacceptable to construe the term "psychological stressor" as being reasonably interpreted to include a situation in which surgery under general anesthesia is performed. Looking at any of Mikawa's figures, it can be seen that a first sample was taken before anesthesia to act as a first point for looking at neutrophil changes during the whole peri-operative period. But it cannot be ignored that the second sample was taken during surgery. This immediately excludes any information being revealed about psychological stress in the pre-operative period (or even post-operative period).

Applicants' present claim 1 also clearly sets forth the significant features which distinguish their invention over all documents of record concerning neutrophil studies. It is unambiguous from the present claim 1 that it concerns a method directed at determining the effect of exposure to psychological stress. This cannot be confused with surgical treatment. It is also unambiguous from the present claims that neutrophils are used as biomarkers for coping capacity (stress effect) without isolation from whole blood samples. It is not acceptable to stretch claim language to a degree that a person skilled in the art would not countenance.

Claims 1-2, 12-14 and 16-17 were rejected as allegedly unpatentable over Mikawa (Can. J. Anaesth. 40:1162-1170, 1993) in view of Carlson et al. (U.S. Patent 6,319,953). Applicants traverse.

The proposed combination of Mikawa and Carlson is no better than the preceding obviousness rejection. One merely has disjointed teachings, which even when combined, do not render obvious the claimed invention. The fundamental deficiency of Mikawa remains as discussed above. It is not cured by noting that others had proposed whole animal models for anxiety drug screening. When samples are taken, how they are processed, and more importantly the comparisons made to quantify coping capacity were not taught or rendered obvious by the cited documents.

Withdrawal of the Section 103 rejections is requested because the claims would not have been obvious to one of ordinarily skill in the art when this invention was made.

Conclusion

Having fully responded to the pending Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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